A STUDY of OVULINIA AZALEAE, WEISS,
in SCOTLAND and an ACCOUNT of the
petal blight it causes in
RHODODENDRONS.

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I N T R O D U C T I O N

The presence of a petal blight was first observed in October, 1950, when black sclerotia were found in withered petals of the hybrid rhododendron "Purple Splendour" at Hareshawmuir Estate, Ayrshire. The bush was noticeable because the dead flower trusses remained on the plant instead of falling at maturity as was the case with uninfected blooms.

The sclerotia were similar in appearance to those described on the petals of azaleas and rhodo--dendrons when attacked by the fungus Ovulinia azaleae, Weiss, in America. (Weiss 1940, Phytopath. XXX). This fungus however, has not been recorded in Britain and only once on hot-house azalea hybrids in Europe (Terrier 1951, Rev.Hort.Suisse XX111).

A study of the Scottish petal blight was made over a period of 2 years to see if the disease was identifiable with the American blight of rhodo-dendrons.

The work included observations of the fungus in nature, its behaviour in culture and attempts to obtain apothecia. A series of infection experiments with a wide range of rhododendrons was also carrid out.

A culture of Ovulinia azaleae was requested from the Type Culture Collection of America so that a comparison with the Scottish fungus could be made. An examination/

/examination of the fungus received was made in culture and its pathogenicity to rhododendron flowers was tested.

In the course of the work it was soon found that Botrytis species were always present in dying rhododendron petals, therefore isolates from conidia were obtained from petals which also contained the cup shaped sclerotia of the petal blight. The Botrytis was studied both in culture and in nature if it was found growing on flower parts of rhododendron, to make certain the petal blight could not be attributed to a strain of Botrytis.

Host Plants and Localities

To date, the fungus has been observed to effect June flowering rhododendron hybrids in North Ayrshire and along the Solway coast from New Abbey to Stranraer. Two bushes of the August flowering Rh. discolor have been seen infected with the disease also.

The material for the study of the fungus was collected from hybrid rhododendrons at Hareshawmuir which were identified as "Purple Splendour" and "Pink Pearl". Later, material was collected from a number of hybrids at Barnbarroch, Dalbeattie.

The bush of "Purple Splendour" was planted in 1900 and is 8 ft. high; for some years it has not made good growth. Petal blight was recorded on it in 1950 but no disease was seen in 1951.

The bush "Pink Pearl", which was planted in 1917, grows about 6 ft. away from that of "Purple Splendour" and is separated from it by a grass path. The "Pink Pearl" was infected in 1951.

The hybrid bushes at Barnbarroch were planted at the beginning of the century and have been observed to bear infection for the past three years. In fact, the fungus appears well established along this part of the Solway coast.

The parentage of hybrid rhododendrons is difficult/

/difficult to trace, but that of "Pink Pearl" is thought to be a hybrid between "George Hardy" and "Broughtonii" and was introduced by the firm of Messrs. Waterer, Ltd., in 1897. It is, therefore, not an original hybrid between two species. The parents of "Broughtonii" are said to be Rh. luteum (Sweet) and a rhododendron of the Arboreum section. The parentage of "George Hardy" has not been traced. "Purple Splendour" is not an original cross between species either, and its origin has not been found.

EFFECT ON HOST PLANT

The first indications of petal blight are small discoloured spots on the corolla lobes. These are rusty brown on white flowers and colourless on coloured petals. The spots increase in size as the fungus grows. Browning and collapse of the tissues follows as the mycelium spreads from the point of infection.

The ascospores germinate under moist conditions on contact with the petal. The germ tube penetrates the cuticle at any point on the petal and does not necessarily enter by a stoma pore. The fungus is capable of piercing the cuticle of perfectly healthy, undamaged tissue requiring no wounded surface to gain entry.

Once inside the petal, the fungus spreads through the tissue by branching mycelium. The hyphae tips grow across the cells and through the cell walls, as well as in the intercellular spaces. The mycelium becomes most abundant in the thin spreading lobes of the corolla. The small, spirally thickened vessels are not disorganised and the hyphae are not observed to enter or grow through them.

The fungus attacks the cell walls of the parenchymatous tissue within the petal, the cells lose turgidity and the protoplasm disintegrates. The petal colour/

/colour is lost due to precipitation of the pigments within the cells. Eventually the internal structure of the petal is entirely disorganised and the mycelium exists on the break down products. The vessels and the cuticle at the outer and inner face of the corolla are the only parts which preserve their original structure.

The flowers at this stage are completely brown and hang limply round the stigmas, adhering together in the truss in the collapsed condition. It is typical of the disease that the flowers attacked by petal blight nearly always remain on the bush in this position becoming quickly dried by sun and wind, thus an infected bush may be identified months after an attack by the dead flower trusses still attached to the branches.

Soon after the petals collapse, clear blister like spots appear in the petal tissue. These gradually darken and eventually black, convex sclerotia are formed. They may remain in the petal tissue for a number of months, but sooner or later they drop out leaving a clear cut hole in the withered petal.

There is no evidence to show that the fungus attacks any other part of the rhododendron except the petals. However, if the attack is severe, the disease causes an untimely collapse of the flower trusses and ruins the bushes for decorative purposes.

TREATMENT OF NATURALLY INFECTED MATERIAL TO PRODUCE APOTHECIA.

The early attempts to isolate the fungus from dead petals or sclerotia were unsuccessful and it was not until May, 1951, that apothecia began to form on infected material and pure cultures of the fungus could be made from ascospores.

Freezing the sclerotia for 4 months in damp silver sand, followed by a period in the light at room temperature did not effect germination. Neither did treatment in the dark at room temperature.

The original sclerotia were again frozen the following winter for 3 months and then placed in the laboratory window, but no germination occurred.

Naturally infected blooms containing sclerotia were similarly frozen during the winter of 1951. After a period of 6 weeks in the laboratory window, apothecia grew both from sclerotia and from hyphae in the dead petals in the month of April.

Some of the material was left unfrozen and 3 apothecia grew from dead petals in November of the same year, from which further isolations were made.

THE FUNGUS IN CULTURE

The fungus was grown on the following media in test tubes in an attempt to obtain the spore stages.

Malt agar

Malt agar: 10% normal strength

+ trace of peptone

Potato dextrose agar

Oat meal agar

Yeastrel agar

Broad beans

Petals of Azalea indica hybrid

Petals of Rh. impeditum on oat agar

on clear agar

Malt agar + $\frac{1}{500,000}$ part of -

Aneurine hydrochloride (vitamin B 1)

Glycene

Biotin

Creatin

Czapek glucose + $\frac{1}{500,000}$ part of -

Biotin

Aneurine hydrochloride

The fungus was also grown in petri dishes on vegetable media as follows -

Wheat kernels

Barley kernels

Cultural/

Cultural Conditions

All the test tube cultures were kept on the laboratory shelves at room temperature usually about 15°C. The petri dishes of wheat and barley kernels were placed in the incubator in the dark at a temperature of 20°C.

During the summer of 1951 the original cultures were maintained in the refrigerator at 5°C in darkness; no particular change in the growth was noted.

Cultures grown in the dark did not appear to be different from those grown in the light.

Preparation of Pure Cultures

Ascospores were obtained from apothecia fixed under the lid of a petri dish containing clear agar. The lid was rotated every half hour and the spores were thus evenly distributed over the surface of the agar.

More spore cultures were made from single ascospores cut from the agar by a "dummy objective". A number of polyascospore cultures were also made.

The original cultures were grown from an apothecium appearing on dead petals of "Purple Splendour" in May, 1951. These had been collected in April from under the bush and placed in a glass dish in the laboratory window.

Later cultures were obtained in November, 1951, when a few apothecia grew from dead petals of "Pink Pearl"/

"Pink Pearl" collected in summer.

In all 14 monospore cultures were made and 6 mass ascospore cultures and these were used for subculturing the fungus onto the different media. From these initial isolations the behaviour of the fungus in culture was observed.

Growth of the Fungus in Culture

The Mycelium. On the media used the fungus produced a short, tough mycelial mat. Colour: smoke grey to hair brown (Ridgway 1912) turning darker with age. There is no perceptible zoning but as the culture grows old, the medium darkens. Growth rate is not rapid: the mycelium requires 3 weeks to cover the surface of a petri dish (diam. 3½") when grown on steamed barley kernels.

Surface growth is more active on malt agar which has had additions of amino-acids; growth rate is also increased.

The young hyphae are colourless, septate and densely protoplasmic. The mature hyphal walls are Natal brown. In these cultures the hyphae averaged 3-4 μ in diameter. Growth is characterised by frequent changes in direction causing an angled appearance. Looping is also seen especially in young hyphae.

Besides the normal hyphae, thick black rhizomorphous/

rhizomorphous mycelium appears in some cultures. These measure about 12 to 20 μ across.

The fungus tends to be short-lived in culture.

Conidia. None has been observed to form from ascospore isolates on any of the media used.

Microconidia. Microconidia have been found growing in old malt agar cultures on wheat kernels. They are produced in tufts on branched spermatophores, the ultimate branches of which are clavate.

Sclerotia. Sclerotia have not been induced to form in the typical manner as found in the natural state. Most sclerotial material was formed on vegetable media such as wheat or barley kernels. In these petri dishes sclerotial material began to form a few days after inoculation with mycelium. Rings of black stroma appeared on the reverse side of the dishes, these merged as the medium darkened.

In the other media in test tubes, the sclerotial formation was confirmed to a black line round the edge of the slope, 1 - 12 mm. thick.

Apothecia. The Drayton method (Drayton 1934, Mycol. XXVI p.46) has been followed, the fungus being grown on wheat or barley kernels. Apothecial fundaments 6 mm. in height formed after three weeks at room temperature, following 3 months of freezing in a refrigerator. No discs matured, however.

DETAILED DESCRIPTION OF FUNGUS

APOTHECIUM

Measurements. Cup: 1 - 3 mm. in diameter.

Stipe: Typically 3 - 5 mm. long

0.5 mm. thick.

Colour. Cup: Brussels brown

Hymenial layer: ochraceous - buff.

Stipe: Raw umber.

The apothecia arise from the sclerotia or from a stroma inside the dead petal. The cup is at first obovate on the stipe; expanding to cyathiform, flat at maturity. The apothecium and stipe have a granulose appearance at first becoming glabrous when mature. The stipe may be considerably longer than the measurements given if growth occurs at low light intensities.

HISTOLOGY OF APOTHECIUM

An examination of the histology was made from paraffin wax sections cut at 5 and 7μ in thickness. The material was fixed in Karpechenko's A. & B. solutions. The slides were stained with Iron Haematoxylin and fast green.

The tissue systems are clearly differentiated in the cup and stipe.

The classification of the various tissue systems is taken from Starbäck's paper (1895 Bihang Till I. Svenska vet. Akad.Hand Lingar.Band 21 Afd.111 No.5)

The/

The asci and paraphyses are developed from a hymenial layer consisting of "textura intricata." Below this the internal tissue of the cup is looser in construction and is formed of "textura oblita". The wall of the cup is composed of "textura globulosa".

The stipe is constructed of separate strands of hyphae which are septate and loosely bound together; the tissue system is "texture porrecta".

There are small globular outgrowths produced at intervals from the surface of stipe and cup; these are hyaline and give the young apothecium a granulose appearance.

The ascospores are uninucleate when mature. When about to leave the ascus the ascospores move from the uniseriate position and lie paired in the top half of the ascus.

ASCUS

Measurements. 59-72.8μ x 4-6.3μ
average 65.8μ x 5μ

Colour. Hyaline.

The ascus is cylindrical; the apical plug does not stain blue with iodine. Contain 8 spores typically; occasionally 4 spored.

ASCOSPORES

Measurements. 7-11.2μ x 3.3 - 4.5μ
average 8.7μ x 4.0μ

Usually 8 per ascus, uniseriate, elliptical, hyaline. One celled, developing a septum on germination. Germination usually by one polar germ pore.

PARAPHYSES/

PARAPHYSES

Cylindrical, unbranched, septate.

CONIDIA.

Measurements. 25-60 μ x 20 - 34 μ
average 40 μ x 26 μ

Colour. Hyaline.

Round to elliptical, produced from short side branches or terminally on the hyphae. A basal cell is formed below the conidium and this remains attached to the spore when the conidium separates from the mycelium. Germination by apical pore.

MICROCONIDIA

Measurements. 2.3 μ - 3.3 μ
average 2.88 μ

Produced on branched spermatophores. Ultimate branches clavate. Microconidia globose, hyaline produced in basi-petal succession. Description from pure culture.

SCLEROTIA

Measurements. 2-5 mm. x 2-10 mm.

0.5 - 1.0 mm. thick.

Colour. Black.

Typically rounded to elliptical, concave. Verrucose on the convex side, smooth on the concave side. Clear, blister-like when forming, becoming brown and opaque; black at maturity.

Cortex narrow, black; medulla white formed of a large thick, oblong to reniform hyphae, embedded in

a/

/a glutinous matrix. The medulla darkens with age. No host tissue is descernible within the sclerotium.

THE LIFE CYCLE

During the month of June and coinciding with the flowering of the host plant, apothecia develop from sclerotia and withered petals which have over-wintered beneath the bush.

The apothecia may arise directly from the sclerotia or from a stroma within the previous year's petals.

The dead flowers are often buried beneath a layer of fallen leaves and detritus, but a percentage of apothecia manages to clear its head above this layer and discharge ascospores into the air.

The ascospores are carried in the air currents and some fall on the opening flowers of the host plant. Here they germinate, and the mycelium enters the petal tissue.

The conidia develop on the surface of the petals during the next few weeks and have been found on late flowering bushes up to the end of July.

The formation of microconidia takes place after the production of conidia and can be found from mid-June onwards. They are borne on spermatophores among the surface mycelium on the petals.

The/

The sclerotia begin to form in August when they are seen as blister-like areas in the dead petals. Gradually they darken and become solid concave bodies. They may remain in the petal tissue throughout the winter, or may drop out leaving a clear-cut hole.

The sclerotia do not always germinate the next year; sometimes they remain dormant till the following or some subsequent year.

COMPARISON OF THE SCOTTISH FUNGUS WITH OVULINIA AZALEAE - WEISS

Measurements taken in water unless otherwise stated.

	Scottish Fungus	Ovulinia Azaleae.
Ascus	59-72.8 μ x 4-6.3 μ average 65.8 μ x 5 μ	140-260 μ x 9-14 μ average 180 μ x 12 μ
Ascospore	7-11.2 μ x 3.3-4.5 μ average 8.7 μ x 4.0 μ	10-18 μ x 8.5-10 μ average 16.3 μ x 9.3 μ
Colour	Hyaline	Hyaline
Conidium + basal append.	25-60 μ x 20-34 μ average 40 μ x 26 μ	40-60 μ x 21-36 μ average 50 μ x 18 μ
Colour	Hyaline	Hyaline
Microconidia	2.3-3.3μ(av:2.88 μ) measured in lacto-phenol	3.0-3.5 μ
Sclerotium	2-5 x 2-10 x 0.5-1.0 mm.	2-5 x 3-10 x 0.5 - 1.5 mm.
Colour	Black	Black
Apothecium	Disc 1-3 mm. diam.	2-5 mm. diam.
Stipe	3-5 x 0.5 mm. typically.	2-3 mm. typically.
Colour of cup	Brussels brown	Tawny olive to snuff brown
" " disc	Ochraceous - buff.	Russet to walnut brown
" " stipe	Raw umber	Clay at base, cinnamon above.
Hyphae	8-12 μ wide	8-12 μ main strands
Colour	Natal brown walls	?

INFECTION EXPERIMENTS

OUTDOORS SUMMER 1951

During June and July some infection experiments were made on rhododendron flowers protected by cellophane bags. Some of these infections were made by placing mycelium from a mono-ascospore culture on the petal in a drop of water. Others were made by suspending an apothecium above the flowers for 24 hrs.

The experiments did not appear to be very successful at the time owing to the many contaminants which appeared on the dying petals, so they were discontinued after 7 had been carried out.

After the trusses were dead, however, petals from 4 of these experiments were placed in damp moss in honey jars. Small sclerotia appeared in the petals by August in 3 of the jars. In November these were placed, together with the petals, in the refrigerator for a period of 4 months. After the freezing the dishes were placed in the laboratory window at room temperature. After 6 weeks apothecia grew from the dead petals.

Of the 3 successful experiments, one, with Rh. ponticum, was infected by an apothecium growing from a sclerotium; another, with "Purple Splendour", from an apothecium growing from petal tissue, and a third, with "Purple Splendour", by mycelium from a mono-ascospore culture.

Flowers/

Flowers from the rest of the 7 experiments were not kept after being examined for the conidial stage which was not identified.

Apothecia from the experiment with Rh.ponticum were used for reisolating the fungus and growing it in pure culture. The germination of the spores and growth of mycelium appear identical with the original isolates. (Koch's 4th-postulate.)

INDOORS WINTER & SPRING 1951-1952

During the winter months some 50 infection experiments have been performed on a variety of rhododendrons and azaleas in an attempt to obtain the conidial stage.

Method.

The apparatus used is adapted from a method evolved by Weiss. (Weiss & Smith 1940 Phytopath XXX p. 447) and used by him for infection experiments with O.azaleae in America.

In this case 1 lb. glass "Kilner" jars were fitted with small test tubes of water; these were stuck to the inside wall of the jars with plasticine. A filter paper was placed on the floor of the jar to absorb excess moisture. The light was cut down to avoid condensation inside the glass by covering the jar with black paper except for a strip 2" wide down one side. A flower of truss of small flowers placed in the/

/the test tubes within the jar remained fresh up to 3 weeks at laboratory temperature.

List of Rhododendrons used.

Number: denotes number of separate experiments carried out.

\+ : denotes some infection took place.

NAME	NO. OF EXPERIMENTS.	
Rh. impeditum.Balf.f. & W.W.Sm.	3	+
Azalea indica hybrids.	20	+
Rh. Javanese Hybrid.	2	+
Rh. obtusum var Kaempferi (Planch.) Wils.	3	
Rh.chrysodoron (Tagg MS) Hutch.	1	
Rh.moupinense. Franch	2	+
Rh.argenteum. Hook.f.	3	+
Rh.lukiangense. Franch	5	+
Rh. Yedoense var poukhanense (Lev) Nakai	1	
Rh.rubiginosum. Franch	1	
Rh.cuneatum. WW.Sm.	1	
Rh.neriiflorum. Franch	1	
Rh. Thomsonii hybrid	2	+
Rh. Morii. Hayata	2	

All infections were made by placing a drop of water containing macerated mycelium directly on the petals.

The list is given in the order in which the flowers became available in the glass houses and later outdoors/

/outdoors at the Royal Botanic Garden, Edinburgh.

To facilitate examination the petals were cleared in a mixture of lactic acid and phenol in equal quantities; clearing usually took 24 hours. They were then washed in water, mounted in lacto-phenol and stained with cotton blue.

DETAILS OF RESULTS

Azalea indica. These flowers were kept from one to three weeks before examination. In 5 out of the 20 flowers a small patch developed round the infected spot; these were brown in the 2 white flowers and colourless in the 3 coloured flowers.

Javanese rhododendron hybrid. A brown patch appeared on the white corolla after two weeks.

Rh. impeditum. Discoloured spots developed in 6 days.

Rh. moupinense. Discoloured spots appeared in 12 days.

Rh. argenteum. Brown patches formed in one week.

Rh. lukiangense. Out of the five flowers infected one formed a discoloured patch after 3 weeks.

Rh. Thomsonii hybrid. Brown patches formed after 2 weeks.

Microscopical examination of all these petals showed only mycelial development, except for one flower of Azalea indica and two of Rh. lukiangense. These flowers had round spore-like bodies in the mycelium resembling/

/resembling conidia in shape but smaller, about 10 μ across, and no basal appendage could be determined. The hyphae on which they grew were very fine in texture.

THE FUNGUS COMPARED WITH BOTRYTIS CINEREA. PERS. EX. FR.

As Botrytis conidia regularly appear on dying rhododendron petals and because this fungus also forms sclerotia towards the end of the season, a careful study was made to see if the petal blight could be attributed to a strain of Botrytis.

Botrytis cinerea in culture.

The fungus was isolated from petals containing the cupulate sclerotia associated with petal blight. The cultures were obtained from conidia; apothecia were never observed in nature.

The Botrytis was grown on a malt agar medium at room temperature. Growth was rapid and the cultures formed masses of typical conidia in a few days.

After 2 weeks sclerotia began to form round the edge of the agar slope. These were very irregular in shape and clearly defined.

Microconidia were produced in the cultures when they were 2 months old; they began to form when the surface mycelium darkened with age.

Compared with petal blight fungus the behaviour of Botrytis cinerea differs in the following ways when grown in culture:

1. The conidial stage is produced freely. None was found in the ascospore isolates of petal blight fungus in a wide range of media.

2./

2. Sclerotia form readily and appear distinct from the medium. In culture, the petal blight fungus does not produce definite sclerotia, but forms a stromatoid rind on the surface of vegetable media i.e. wheat and barley kernels and broad beans. On agar slopes sclerotial material is confined to a black line round the edge of the slope.

3. Microconidia are formed in quantity in staling cultures. With the petal blight fungus microconidia are much less abundant and were only found in a few of the many cultures made.

Botrytis sclerotia formed in culture were treated by the Drayton method (Groves and Drayton 1939, Mycol.XXX1 p. 485) in an attempt to obtain the perfect stage, but no apothecia formed.

Botrytis cinerea as it effects rhododendron flowers.

Conidial infection in nature is common on rhododendron flowers. The fungus gains entry into the petal tissue through the smallest abrasion.. Infected corollas collapse within a few days and typical conidiophores are produced over the surface of the petal.

Sclerotial development in petals, however, is not at all common. The sclerotia are generally confined to the firmer tissues of capsule and pedicel.

Examination of hundreds of dead rhododendron petals/

/petals occasionally revealed thick, black sclerotia with irregular shape.

A collection of these sclerotia was made and placed on damp silver sand in the refrigerator for 3 months. They were then placed in the laboratory window. After 2 weeks Botrytis conidia grew out of the sclerotia in quantity.

Freezing of sclerotia naturally produced by the petal blight fungus has never formed any kind of conidia. The only spores seen on these sclerotia have been those of recognisable contaminants such as Trichoderma and Trichothecium species. No botrytis-type of condidia was ever seen growing from them.

Spore measurements, taken in water, for the strain of Botrytis cinerea used in these studies were as follows:

Conidia:	10-13.3 μ long x 5-7.6 μ wide average 10.8 μ x 6.3 μ
Microconidia:	2.6 - 3.3 μ diameter average 2.9 μ

STUDY OF CULTURE NO. 10935 from THE TYPE CULTURE COLLECTION. U.S.A.

A culture of Ovulinia azaleae. Weiss. was requested from Dr. Weiss in order to compare it with the Scottish petal blight fungus. He kindly sent a culture of the only available material at the time. This fungus was isolated by a colleague from a local source (Washington D.C.) as he had not got any of his own original isolates. It had not been tested for pathogenicity at his department and Dr. Weiss added that it might possibly have been contaminated with a Botrytis. It is not known whether the culture originated from ascospores or from conidia.

The fungus was received in January, 1952. The medium was a neo-peptone glucose agar. The mycelium formed a dense surface mat olive buff to buffy brown. Examination showed the fungus was producing masses of microconidia. In mounts made from the culture a few Botrytis-like conidia were present.

The fungus was sub-cultured on to malt and oatmeal agars in test tubes which were kept at room temperature. Petri dishes of wheat kernels were also inoculated with some of the mycelium; these were kept in the dark in the incubator room at 20°C.

Growth of the mycelium on all these media

was/

/was very rapid compared with the Scottish petal blight; the surface of a 3½" petri dish was covered in 6 days.

None of the cultures formed recognisable sclerotia, nor was there any darkening of the medium with age.

After 2 months the petri dishes were placed in the refrigerator for a period of freezing, followed by 3 weeks in the laboratory window, but no apothecia formed.

Infection Experiments.

A series of infection experiments with living rhododendron flowers was carried out with this fungus. The same method and apparatus was used as with the Scottish fungus in the indoor infection experiments.

A list of the rhododendrons and azaleas follows with the number of separate experiments on each species or hybrid appended:

NAME	NO. OF EXPERIMENTS
Azalea indica hybrids	5
Rh. moupinense. Franch	6
Rh. chrysodoron (Tagg ms.) Hutch	1
Rh. argenteum. Hook. f.	2
Rh. leaucaspis. Tagg.	1
Rh. reticulatum D.Don. apud G.Don	1
Rh. Thomsonii hybrid	2

The/

/The petals were cleared before examination as described for the other indoor infection experiments.

The results indicate that the fungus is capable of damaging rhododendron petals by causing collapse of the tissues. A rapid browning follows and is observed two days after inoculation. The mycelium spreads from the point of infection and grows both internally in the tissue and on the surface of the petal.

This rapid browning effect was observed in all the experiments except with the hybrid Rh.Thomsonii, but spores of the Ovulinia-type could not be found. Sometimes the mycelium formed branches resembling conidiophores of the botrytis-type. Spores were not developed, however, by the time the corolla had completely collapsed; this condition was generally attained in two weeks from time of infection.

In the experiments with Rh.chrysodoron and Rh. argenteum microconidia were found.

A flower of Rh. argenteum was kept two months in a damp chamber, but no sclerotia were developed.

Footnote: Measurements of microconidia: 2.8 - 3.0 μ

FUNGI ASSOCIATED WITH DYING PETALS OF RHODODENDRON

In the course of the investigation of the fungus causing petal blight in rhododendron hybrids, a number of fungi were found commonly associated with dying rhododendron flowers. A brief survey of these fungi may be of value to other workers in this field.

By far the commonest was a fungus referable to Botrytis cinerea Pers. ex. Fr. which invades damaged or bruised petals very quickly and the mycelium travels rapidly through the tissue and cause the collapse of the flower within 3 - 4 days, the typical conidia forming in one week. The mycelium ramifies in the stamens stigma and calyx as well as the petals. The hyphae often form thick walled chlamydospores in the petal tissue, increasing in quantity as the petal withers.

The anthers and pollen of rhododendrons are often attacked by Penicillium species, the mycelium spreading down the filaments and causing the stamens to collapse onto the corolla tube. The fungus then spreads rapidly in the petal tissue. Penicillium species may also cause colourless areas or spots on the edge of the corolla lobes from which typical fructifications grow out.

Trichothecium roseum sometimes appears in the later stages of decay and also grows on the surface of sclerotia formed in the petals by petal blight disease.

Aspergillus/

Aspergillus species are also frequent usually spreading over petals already attacked by botrytis. Species of the *Penicilloides* group are the most common.

After the flowers are quite withered but are kept damp, *Trichoderma viride* regularly appears. At first the fungus forms white fluffy spots on the surface of the petal; these turn green as the spores mature. In the tissue chlamydospores are often formed and they bear a similarity to the conidia of *Ovulinia azaleae*, but they are only half the size and have a thin wall; also the mycelium in which they occur is very fine compared with *Ovulinia* and is hyaline.

A *Phoma* species with typical black walled pycnidia has been found. The pycnidia extrude one celled hyaline spores in irregular masses when crushed. The pycnidia do not arise from a definite stroma within the petal, but are seen scattered over the dead corolla tube.

In the attempts to isolate the petal blight fungus from surface sterilized petals, a number of fungi grew out from the dead tissue onto the culture medium. Again *Botrytis* species were in the majority; besides *Botrytis cinerea*, there was a noticeably different species with thicker, darker mycelium and darker spores. The sclerotia formed from this species were smaller and more numerous in culture as compared with *B. cinerea*.

Other fungi genera identified were *Fusarium* and *Mucor* species.

DISCUSSION.

This fungus causing a petal blight of rhododendron hybrids in Scotland is identifiable with Ovulinia azaleae, Weiss, in spore form and life cycle. The limited material so far available for examination has not been sufficient to make comprehensive spore measurements, but those calculated fall within the size limits of the American fungus. In measuring ascospores it has been found of more value to measure a few spores from a large number of apothecia in order to arrive at the true mean. At present some dozen apothecia have been used for this purpose but the results indicate that this number is not enough.

The average size calculated for an ascospore of the Scottish fungus is smaller than that given by Weiss for O. azaleae. The discrepancy is most marked in the width of the spore; the length of the largest in the Scottish fungus is given as the length of the smallest in the American fungus.

The length of the ascus in the two fungi show an even greater variance, but here again it is deemed necessary to take more measurements from a much larger number of apothecia before the true average size can be ascertained.

Terrier (1951 Rev.Hort.Suisse XX111) omits to give measurements for asci of the fungus he found on hot-house/

/hot-house azalea plants in Switzerland, but his measurements for conidia, 20μ - 60μ, show a wider range than those given by Weiss. The measurements of conidia in the Scottish fungus come within the range stated by Terrier, but are smaller than the measurements of those given by Weiss. As Weiss did not give a name to the conidial state of the American fungus, Buchwald (1949 Studies in the Sclerotiniaceae No.32) proposes to call it Ovulitis. He considers it practical to have a distinct name for the conidial state especially in parasitic fungi.

In his description of O.azaleae, Weiss (1940 Phytopath XXX) stated that the apothecia are formed from sclerotia. In the Scottish fungus it has been found that apothecia may arise from the cup-shaped sclerotia or from mycelium within the dead petals. In fact more apothecia were seen growing from dead petals than were produced from actual sclerotia. The fungus has been isolated from apothecia growing from both substrates and subsequent infection experiments with the isolates have proved that the same fungus is grown from petals or sclerotia.

It is possible that Weiss may have removed the sclerotia from the dead petals when these were placed in damp sand to develop apothecia, in which case the petals may have been overlooked as a possible auxiliary/

/auxiliary substrate from which apothecia might grow.

In addition to the points of variance already mentioned, the American and the Scottish fungi differ in their physiological behaviour.

With O. azaleae, Weiss found no difficulty in producing the conidial stage on suitable living hosts; the spores growing within three days of infection with mycelium, and within a week after inoculation with ascospores. Mycelium grown in culture from ascospores, of the Scottish fungus, however, does not readily infect flowers in like experiments. The spore-like bodies found in some of the experiments after the flowers had been kept from 3 - 4 weeks were presumed to be chlamydospores. Infection is more readily attained when ascospores are arranged to fall directly onto flowers, but in the outdoor experiments carried out in the summer of 1951, the conidia could not be found and it was not until sclerotia began to form that the disease was recognised.

The main difficulty in observing the conidial stage in infection experiments is the early entry of Botrytis species which invade the petal tissue far more quickly than the petal blight fungus.

However, in nature, conidial development is rapid when conditions of weather favour the growth of the/

/the fungus, that is to say when temperature and humidity are high. In June 1953 there were sheltered plantations of rhododendrons near Dalbeattie where it was difficult to find an uninfected bush. On the other hand in 1952, conditions did not favour conidial development in the same locality and only odd bushes bore infection.

The conidial stage of O. azaleae has not been obtained in culture, either in the course of this work or that undertaken by Weiss. It is concluded that the fungus requires particular substances in the petal to attain its true form and sporing activity; on artificial media the mycelium is never so robust and appears to lose its capacity to infect live tissue and to produce conidia.

The life cycle of the Scottish fungus agrees with O. azaleae as far as is known. A point yet to be determined is whether the minute spines found by Weiss are also formed on young sclerotia in Scotland. Weiss suggests these spines may be receptive organs for the microconidia and therefore have a sexual significance and be similar in function to those produced by Sclerotinia gladioli in culture. (Drayton 1934, Mycol. XXV1 p.46.) It has not been extablished whether O. azaleae is heterothallic or homothallic. Work on the sexuality of the Scottish fungus is not yet complete,/

/complete, but in culture no antagonism between pairs of monascospore isolates could be seen.

The histology of the apothecium, on which Nannfeldt (1932 Nova Acta Regiae Soc.Sci.Upsaliensis Ser.1V 8 (2)) bases his classification of the inoperculate Sclerotiniaceae, has not been described for O. azaleae, so no comparison can be made with the Scottish fungus.

The time of apothecial development in Spring differs in the two fungi. In America, the States where O. azaleae is recorded lie between latitude 30° and 39°. In the southern part of its range the apothecia appear in February and are coincident with the opening of the flowers of Azalea indica; in the northern limits of the range apothecia do not appear until April and are concomitant with the later blooming of azaleas in these States.

In Scotland the stations recorded for petal blight lie between latitude 55° and 55.5° . The apothecia here are coincident with the blooming of the June flowering hybrids and Rh. ponticum.

The more northern distribution of the Scottish petal blight may well account for the later development of the perfect stage. It is reasonable to conclude that apothecial development will be governed by local climatic conditions prevailing in Spring in both/

/both countries, just as the flowering time of rhododendrons and azaleas is affected from year to year.

In America O. azaleae was first recorded in 1931 near Charleston, South Carolina. Since then it has spread to all the South Eastern and Gulf States from Maryland to Houston, Texas. Harris (1941 Plant Disease Reporter XXV) reported isolated outbreaks of the disease near Los Angelos. The fungus is thought to have been introduced from the Orient about 1920 when large consignments of plants known horticulturally as Indian and Kurume azaleas were imported.

Weiss, in a written communication to the author, states that the disease has been observed in Japan by visitors to that country who have brought back photographs of infected plants as evidence.

A search of the literature, however, shows no published record of the disease in Japan or in any other part of the Orient. From Europe there is a single reference to O. azaleae found on hybrid plants of Azalea indica under glass (Terrier 1951). These had been imported from Belgium in 1948, but a subsequent search for the fungus by mycologists in Belgium and Dutch nurseries has been unrewarded.

As the rhododendron hybrids on which the Scottish blight occurred in 1950-51 are long extab-lished bushes growing in two isolated gardens, it seems unlikely/

/unlikely that the fungus causing the disease is of recent introduction. It has already been proved by an outdoor infection experiment that the fungus will grow on Rh. ponticum; this opens up the question whether this particular fungus could have been brought to Britain with Rh. ponticum from Europe, spreading later to hybrids having a similar flowering period.

The parentage of the rhododendron hybrids "Pink Pearl" and "Purple Splendour" have been difficult to trace as neither are original hybrids made directly from species. At the time these hybrids were produced at the end of last century, records of the parentage were not required for publication and the nurseries interested in creating new hybrids kept such information strictly secret. Thus, through time, it has become lost. As far as can be traced these two hybrids are descendants from crosses originally produced from Oriental and Eastern European species; not American.

In America O. azaleae was first recorded on plantings of Indian azaleas whose derivation is traced mainly to Rh. Simsii Planch. Rh. pulchrum, Sweet. and Rh. mucronatum G. Don, and on Kurume azaleas derived Rh. obtusum (Lindl) Planch.

These species mentioned are also susceptible to attack as well as some other rhododendron species of oriental origin when grown in contact with the fungus. The species on which blight has been recorded include Rh./

/Rh. indicum, (L) Sweet, Rh. japonicum(A.Gray) Suringar., Rh. obtusum var. Kaempferi, (Planch) Wils., Rh. molle (Blume) G. Don. and Rh. Yedoense var poukhanense (Lev.) Nakai.

Some of these species were available for infection experiments with the Scottish fungus but the results were not convincing. Much more work is needed in experimental infection of different species and hybrids of rhododendron to determine the host range of the fungus.

It is unfortunate that the only available culture of Ovulinia azaleae did not prove to be typical, as first hand examination of material would have been helpful in identifying the Scottish fungus. The culture received is certainly pathogenic on rhododendron petals, but as no conidia or sclerotia could be induced to form typical of O. azaleae it is concluded the isolate is not that fungus. Identification is not possible without the formation of some kind of spore; up to date these have not matured in infection experiments.

The study of the strain of Botrytis isolated from petals containing sclerotia of the petal blight gave conclusive evidence that the disease was not attributable to Botrytis. However, this fungus is the first to enter damaged tissue from whatever cause and

was/

/was always present in every suspected infection by petal blight, which complicated identification of this fungus under natural conditions.

The germ tubes of Botrytis conidia seem unable to penetrate undamaged living tissue as Brooks (1908) confirmed in his work with lettuce plants. O. azaleae on the other hand does enter perfectly fresh, undamaged petal tissue. (Weiss and Smith 1941, U.S. Dept. of Agric. circular No.556.) (Terrier, 1951.)

Attention is drawn, at this point, to the delicate texture of rhododendron petals which bruise extremely easily. Even heavy rain drops may cause "spotting" and marks left by insects visiting the flowers are often seen on the corolla.

SUMMARY

A fungus similar to Ovulinia azaleae, Weiss, causing a petal blight of hybrid rhododendrons in Scotland is recorded.

The behaviour of the fungus in culture and in nature is described and a histological description of the perfect stage is included. The result of a number of infection experiments have established that the fungus is pathogenic to some rhododendrons.

The Scottish fungus is compared with the American O. azaleae and it is concluded that it is identifiable with this fungus.

A number of fungi commonly associated with dying rhododendron petals are described; one of them Botrytis cinerea occasionally forms sclerotia in petals but is not found to cause the petal blight disease.

A culture received from America has not proved typical of O. azaleae after being studied in culture and used for infection experiments.